

**Research Article**

## **Effect of different freezing methods on drip, texture, microstructure in fresh cheese (paneer)**

Rajib Lochan Poudyal<sup>1</sup>, Manabu Watanabe<sup>1\*</sup> and Toru Suzuki<sup>1</sup>

<sup>1</sup>Department of Food Science and Technology,  
Tokyo University of Marine Science and Technology,  
Minatoku Konan 4-5-7, Tokyo, Japan

\*Correspondence: [mwat@kaiyodai.ac.jp](mailto:mwat@kaiyodai.ac.jp)

ORCID: <https://orcid.org/0000-0002-6027-5853>

Received: September 01; Accepted: December 6; Published: December 09.

© Copyright: Poudyal et al. (2018).



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](https://creativecommons.org/licenses/by-nc/4.0/).

### **ABSTRACT**

Paneer is acid heat milk coagulated soft cheese popular in South East Asia. The shelf life is a major limitation for its utilization; even when kept under refrigeration. Similarly, the texture becomes flaky and fluffy after thawing. Freezing rate effects on the growth of ice crystals as well as microstructure damage that is responsible for the change in the textural quality of the Paneer. In this study, the effect of two-dimensional freezing by static air observed before and after freezing. Effect of freezing on paneer were subject to analyses under slow freezing at -30°C, rapid freezing at -80 °C and supercooling freezing conditions. The quality evaluation carried out by means of drip loss, textural analysis, and microscopic observation. By using this technique, it was possible to decide the supercooling freezing is significantly best method for preserve the paneer to keep required hardness, low drip and small cracks and rupture in a microstructure in compare to another method.

**Keywords:** Supercooling, Freezing, Thawing, Nucleation, Texture.

**Correct citation:** Poudyal, R. L., Watanabe, M., & Suzuki, T. (2018). Effect of supercooling freezing on texture, microstructure in fresh cheese (paneer). *Journal of Agriculture and Natural Resources*, 1(1), 161-167.

---

## INTRODUCTION

Paneer is a variety of soft fresh cheese produced by acid heat milk coagulation popular in the south Asian region. It is used as ingredients materials for culinary and snacks, which can be consumed directly. Ideal quality of paneer is a spongy body, smooth texture and nutty flavor along mildly acidic taste. Paneer is the good source of animal proteins, fats, minerals and vitamins (Goyal & Goyal, 2016). Paneer is the concrete source of animal protein for the vegetarian diet with high nutritive value (Dwivedi *et al.*, 2010). However, it has a short shelf life of 6-7 days in refrigerated condition and only one day in the room temperature. To overcome such short shelf life, freezing is the best method of food preservation for long time storage, which preserves the flavor as well as the nutritional quality of foods (Alizadeh *et al.*, 2007). However, it can reduce the oxidation process in proteins and lipids during storage as well as inhibit undesirable biochemical change. Ice crystals cause microstructure damage during freezing which is responsible for the change in textural quality in fresh cheese. Crystallization of solute and water are the main reason for the undesirable physicochemical change in freezing (Charoenrein & Owcharoen, 2016). Furthermore, frozen paneer of different companies is accessible in the market all over the world. They are high in fat and low in moisture content than loose paneer due to the improvement of microstructure damage by illuminating the ice crystals from water content. Preservation of texture and sensory quality is most important technology for the food products during storage. Researchers have reported that preservation of paneer have been found to be critical.

Although, supercooling freezing is the best method in ambient pressure supercooling and freezing but less research in foodstuffs. Some research were conducted on supercooling freezing of pure water and other liquids; little data are available on the supercooling freezing of the food products. Few studies on supercooling freezing cited in fruits and vegetables like tomato, grape, strawberries, and garlic (James *et al.*, 2001) report that apple can be store 4°C below than freezing point. Whereas tomato, orange, grape follows, the same trend have been reported in the garlic (James *et al.*, 2009) which can be stored below the freezing point -2.7 °C with no freezing. They can able to store for one week at -6 °C without freezing in some trials, After breaking the supercooling there is less damage in the microstructure (Fuchigami *et al.*, 1998). The aim of this research was to monitor the supercooling freezing effect on fresh cheese (Paneer), to analyze the microstructures of ice crystals compare to classical methods slow freezing and rapid freezing.

## MATERIALS AND METHODS

### Sample preparation

Paneer was prepared from the not homogenize milk pasteurize at 72<sup>0</sup>C for 15 seconds processed by Kitsugi Dairy Co., having 3.4% fat ,SNF of 8.2% and pH 6.74. After that paneer are cut into the size of 2×2×2 cm<sup>3</sup> by using a sharp knife, the mass was approximately 8.5 gram. Samples kept in the refrigerator for analysis after measuring the water content and texture for conforming equality in the batch.

### Moisture content and Drip Loss

The moisture content of paneer samples was determined by drying in hot air oven within 1 hour after draining the free water. Drip loss and water content were measure as:

$$\text{Drip loss/Water content} = \frac{(\text{Initial disc weight} - \text{final disc weight}) \times 100}{\text{Initial disc weight}} \dots\dots\dots \text{Eq. (1)}$$

### Supercooling and freezing trials

Numbers of trials made to establish the freezing and supercooling of the paneer. The supercooling was carried out by using (Shibata Co.SMU-053) incubator, Hioki data logger (Hi-Logger series LR8431-20) was used to observe the temperature .The samples were keep inside the incubator set at  $-10^{\circ}\text{C}$ . When all the probe temperature breaks the supercooling then, sample were transferred into  $-30^{\circ}\text{C}$  deep freezer (SYNO Co.SCR-F221G). For the freezing the static air deep freezer of  $-80^{\circ}\text{C}$  (SYNO Co.MDF-C8V1) and  $-30^{\circ}\text{C}$  was used which is also connected to the data logger for temperature observation.

### Textural Analysis

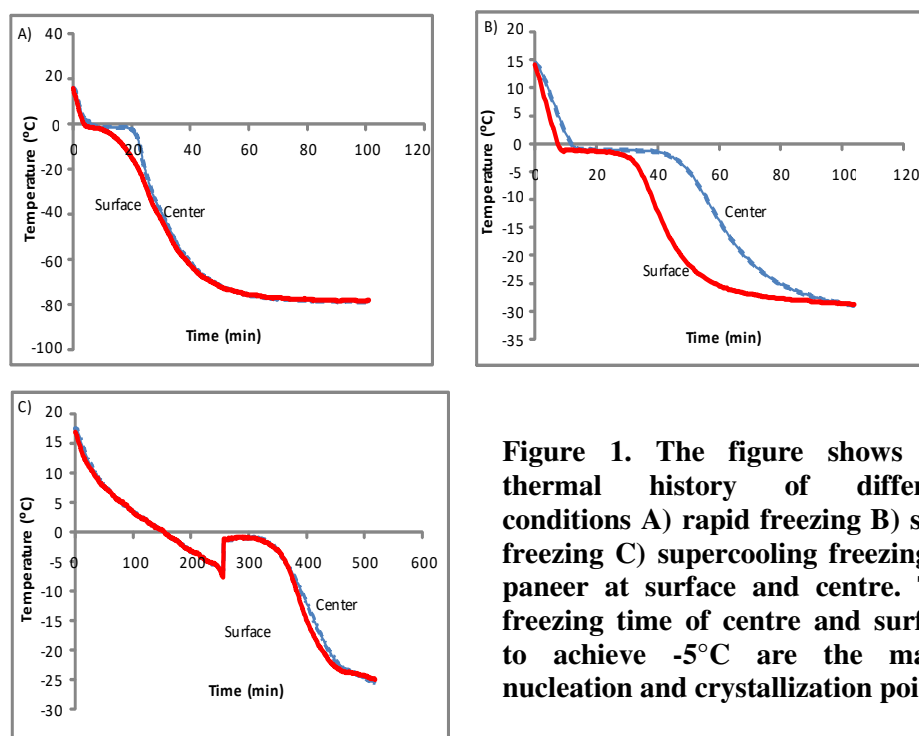
For the textural analysis, hardness is the major indicator to compare different conditional profile in paneer. It was measured by the textural analyzer (TA, XT Plus, Stable Micro system U.K). The parameter setting was the probe P/75, pre-test, and post-test speed was 10 mm/s and test speed was 1 mm/s, final displacement is 80% for original height. The samples were tested after air thawing at  $20^{\circ}\text{C}$ .

### Microscopic observation

Samples were immersed into the liquid nitrogen thus obtain frozen samples were broken into small pieces; the proper piece was selected and keep inside the glue for fixing. The sample were dipping into n-hexane with dry ice for 5 to 10 minutes for solidification of glue, then the samples were stored in  $-80^{\circ}\text{C}$  freezer. For further analysis, sample are cut into  $10\text{ }\mu\text{m}$  slices by using freezing microtome (LeicaCM1850) followed by washing with distilled water and 70% (w/v) ethanol solution. The prepared slides and air-dried before stained with 1.0% (w/v) rhodamine B in 2-methoxyethanol.The microscopic observation was carried out by the Keyence BZ9000 fluorescence microscope at a magnification 100X.

## RESULTS AND DISCUSSION

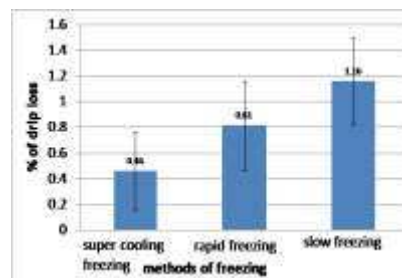
The water content directly effects in ice crystals formed during the nucleation and crystallization process. During this research only the moisture content  $50 \pm 2$  % are accepted. The moisture content is depending upon the temperature of the coagulation type of acid for coagulation and pressing time. Fig.1 A, B, C represents the mean value of the temperature freezing curves of paneer sample during rapid freezing, slow freezing and supercooling freezing condition in surface and center. In slow freezing, as shown in Fig.1, surface temperature appeared to decrease more rapidly than the center temperature, the same trend followed by rapid freezing as well. The time taken to obtain  $-5^{\circ}\text{C}$  by center and surface is significantly different in  $-30^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  but there is no difference in supercooling freezing, due slow and steady cooling and freezing. Similarly, the time taken for nucleation and crystallization in  $-80^{\circ}\text{C}$  is less than  $-30^{\circ}\text{C}$  in surface and center of the same sample. It is coincides in the figure 1(C) due to suddenly, breaking supercooling point where nucleation and crystallization occur at once. Increasing the nucleation and crystallization time, the sizes of ice crystals have also increased which negatively affect in drip, texture and microstructure. Nucleation point or 'metastable limit temperature' (the point at which ice nucleation is initiated) in supercooling was between  $-5.1^{\circ}\text{C}$  and  $-7.5^{\circ}\text{C}$ . This means paneer remained unfrozen in a standard condition below its freezing point at  $-6 \pm 2^{\circ}\text{C}$ .



**Figure 1.** The figure shows the thermal history of different conditions A) rapid freezing B) slow freezing C) supercooling freezing of paneer at surface and centre. The freezing time of centre and surface to achieve  $-5^{\circ}\text{C}$  are the major nucleation and crystallization point.

## Drip loss

The drip loss reflects the structural damage by the ice during freezing and thawing cycle. This result shows the natural water loss after freezing and thawing of paneer. During freezing ice crystals disruption in the casein matrix and casein fat structure, lead to reduce water holding a capacity of the paneer and release the water as a drip. According to Fig. 2, drip is significantly higher in slow freezing than supercooling freezing and rapid freezing due to ice crystals causes more cellular damage in the protein matrix. According to result, the drip loss of slow freezing is significantly higher by 1.16% after 45 minutes of thawing at 20°C whereas the supercooling has only 0.46% it means the freezing causes more damage in the structure of the paneer during the freezing-thawing cycle. Slow freezing process consumed a long time for the nucleation, crystallization, and recrystallization process, which tended to enough growth of ice crystals sizes.



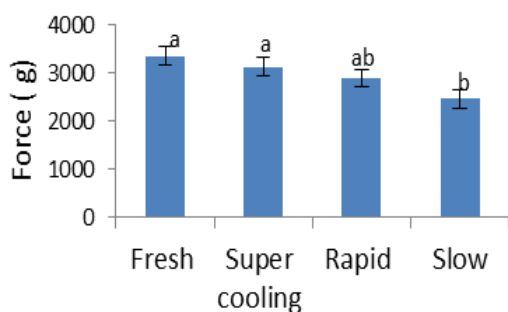
**Figure 2. Drip loss affected by different freezing methods.**

## Texture Analysis

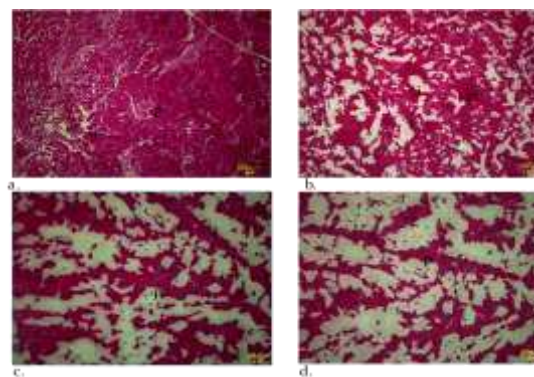
The textural quality is an important characteristic for frozen foods stuff to analyzed for their stability during freezing. The hardness indicates the structural damage caused by the freezing and thawing which softening the paneer, it depends upon the storage temperature (Goyal & Goyal, 2016). All the data of paneer were obtain by the instrumental measurement during texture analysis. Hardness is the significantly higher  $P (\leq 0.5)$  in supercooling freezing (Fig. 3), in comparison to slow freezing. This indicates the higher textural damage occur during slow freezing due to the formation of large ice crystals. The microstructure of casein gets less rupture in case of supercooling freezing so; hardness remains 3139.53g from 3359.19g, whereas rapid freezing and slow freezing remains 2903.77g and 2473.67g respectively (Fig. 3). Supercooling freezing have very short nucleation and crystallization period, the growth of ice crystals is minimum, uniform and small sizes of ice crystals are formed and the microstructural damage become less as result textural quality remain good. However, in normal cycle (-30°C) long time require to be frozen the sample, at mean time nucleation, crystallization and recrystallization occur which directly affect in the hardness after thawing. Furthermore, the rapid freezing has shorter time than slow freezing process that improved hardness in some extent.

### Microscopic Analysis

Fig. 4 (a) disclosed the fresh paneer sample contained water (indicated as white part). White part increased in order to Fig. 4 (b), (c), (d). In the fresh sample, casein molecules are compact, rigid and tightly packed some crack is observed due to freezing in liquid nitrogen also reported by (Fontecha et al., 1996) in ewe's milk cheese. The supercooling freezing realizes the smaller amount of damage in the casein matrix than other freezing methods. The sizes of ice crystals are relatively same and uniformly distributed over the samples (Fig 4b). The intramolecular gap and size of ice crystals are increased simultaneously in rapid freezing and slow freezing in Fig. 4 (c) and (d). The average areas of ice crystals are comparatively small in supercooling freezing than other two methods. In order to rapid and slow process huge amount of casein matrix are tearing and formed irregular shape and size. The area of ice crystals also increases almost all part of the sample.



**Figure 3. The average hardness of the paneer (n=5) sample after cool air thawing at 20°C where supercooling freezing is significantly higher  $P \leq 0.5$  than slow freezing**



**Figure 4. Microscopic observation of frozen paneer: (a) Fresh, (b) Supercooling freezing, (c) Rapid freezing (d) Slow freezing**

According to investigation, supercooling freezing had a good hardness of 3034.32 g in comparison to rapid freezing and slow freezing. Similarly, the statistical analysis of drip loss was appeared to be best in supercooling freezing as low as 0.46% while other remains high as 0.81 % (rapid freezing), 1.16 % (slow freezing). Furthermore, we confirmed a number of small sizes of ice crystals during supercooling freezing in microscopic observation in Fig. 4 (B). Supercooling freezing can expect to improve the hardness, drip loss and microstructure of frozen-thawed paneer. The frozen damage can overcome by this method. Rapid freezing and slow freezing cannot avoid higher damage in microstructure that results in low hardness and high drip loss.



## CONCLUSION

The supercooling freezing investigated as best method for frozen storage of paneer due to its high textural quality and low drip loss. During supercooling rupture in the microstructure are comparatively smaller than another freezing method. Paneer frozen in supercooling freezing was indicate to attribute the smaller ice crystals formed in paneer textures relative to other freezing conditions.

## Author Contributions

RLP designed and performed experiments, analyzed data and wrote wrote the manuscript in consultation with MW and TS revised the article for the final approval of the version to be published.

## Conflicts of Interest

The authors declare that there is no conflict of interest.

## REFERENCES

- Alizadeh, E., Chapleau, N., de Lamballerie, M., & Le-Bail, A. (2007). Effect of different freezing processes on the microstructure of Atlantic salmon (*Salmo salar*) fillets. *Innovative Food Science & Emerging Technologies*, 8(4), 493–499. <https://doi.org/10.1016/j.ifset.2006.12.003>
- Charoenrein, S. and Owcharoen, K. (2016). Effect of freezing rates and freeze-thaw cycles on the texture , microstructure and pectic substances of mango. *International Food Research Journal*, 23(2), 613–620.
- Dwivedi, B., Singh, J., & Yadav, Y. (2010). Effect of type of milk, coagulants with concentration and coagulation temperature on quality of paneer. *The Journal of Rural And Agricultural Research*, 10(1), 4–6.
- Fontecha, J., Kalab, M., Medina, J., Pelaez, C., & Juarez, M. (1996). Effects of freezing and frozen storage on the microstructure and texture of ewe's milk cheese. *Zeitschrift Für Lebensmittel-Untersuchung Und Forschung*, 203(3), 245–251.
- Fuchigami, M., Teramoto, A., & Ogawa, N. (1998). Structural and Textural Quality of Kinu-Tofu Frozen-then-Thawed at High-Pressure. *Journal of Food Science*, 63(6) [Onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2621.1998.tb15853.x](http://Onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2621.1998.tb15853.x)
- Goyal, S., & Goyal, G. K. (2016). Maximizing Shelf Life of Paneer—A Review. *Critical Reviews in Food Science and Nutrition*, 56(8), 1253–1261. <https://doi.org/10.1080/10408398.2012.763157>
- James, C., Hanser, P., & James, S. J. (2001). Super-cooling phenomena in fruits , vegetables and seafoods. [www.researchgate.net/publication/265178903](http://www.researchgate.net/publication/265178903)
- James, C., Seignemartin, V., & James, S. J. (2009). The freezing and supercooling of garlic (*Allium sativum* L.). *International Journal of Refrigeration*, 32(2), 253–260. <https://doi.org/10.1016/j.ijrefrig.2008.05.012>